#6 m.m. 7/30/02

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| Applicants: | Konowalchuk, et al. | ) | |
| Serial No.: | 10/021,533 | ) | Art Unit: 1617 |
| Filed: | December 6, 2001 | ) | |
| Title: | METHOD FOR TREATING AN INFLAMMATION OR LESION CAUSED BY A VIRUS | ) | Examiner: Hui, S. |

To: Commissioner for Patents
Washington, D.C. 20231

**RULE 132 DECLARATION OF DR. JACK KONOWALCHUK**

STATE OF OREGON )
) ss.
COUNTY OF LINCOLN )

I, Jack Konowalchuk, declare:

1. My name is Jack Konowalchuk, and I reside at 1098 N.E. 7th Drive, Newport, Oregon.

2. All of my statements in this Declaration are accurate and true to the best of my knowledge and belief.

3. I am currently a research scientist, a position I have held since 1999, where I am responsible for research activities on the virucidal composition.

4. As background information and as foundation for my statements in this Affidavit, I received a Bachelor of Science Degree from University of Manitoba in 1946, and I received the degree of Doctorate in Microbiology from the Queen's University in 1952. I have over 50 years of experience researching in the virology field.

5. From 1947-1963 at the Defense Research Kingston Laboratories, I was the Scientific Officer respondent for general microbial research.

6. In 1963, I accepted a position with Defense Research Board, Shirley's Bay

Ottowa as Head of Virus group as Scientific Officer responsible for the rapid identification of human viruses.

7. From 1969-1985, I worked at Health and Welfare Canada, Health Protection Branch, Bureau of Microbial Hazards as Head of Food Virology, Research Scientist 3. I planned an developed methods for virus recovery from foods.

8. I have authored or co-authored more than 35 scientific publications in my career and I have presented many papers at numerous proceedings.

9. I am a co-inventor of U.S. Patent Application No. 09/795,279, filed February 28, 2001.

10. I have reviewed the Examiner's final Office Action, Paper No. 10, dated February 12, 2002. It is my understanding the Examiner has determined that the data presented in U.S. Patent Application No. 09/795,279 do not show unexpected results, and that it would have been obvious to combine the agents of the claimed compositions based on the prior art cited in the final Office Action. I do not agree.

11. I have reviewed the references cited in Exhibit B to be submitted with the response to the final Office action.

12. It is my belief that the references cited in Exhibit B demonstrate that lower chain alcohols at low concentrations alone do not have virucidal activity.

13. I conducted the assays summarized in Table 2 of U.S. Patent Application No. 09/795,279.

14. The data presented in Table 2 of the present invention demonstrate that glycolic acid alone is virucidally effective when at a pH at or below 4.0. The data also demonstrate that glycolic acid is not virucidally effective when at a pH above 4.0.

15. I conducted the assays summarized in Exhibit C to be submitted with the response to the final Office action, Paper No. 10.

16. The data provided in Exhibit C summarizes the results of an assay in which various amounts of ethanol (1%, 5% or 10%) were combined with a 0.6% glycolic acid solution and the pH of the mixtures were adjusted to a pH of 2.5, 3.5, 4.0, 4.5 or 5.0. The final solutions were then assayed for virucidal activity against the Herpes Simplex 1 virus in a manner similar to that described in Example 1 of U.S. Patent Application No. 09/795,279.

17. Exhibit C provides supporting data showing the synergistic effect of the acid, alcohol, and pH agents of the claimed invention. The data provided in Exhibit C show that when low concentrations of ethanol (e.g., 1-10%) are combined with glycolic acid at a pH between 2.5 and 4.5, the resulting compositions have virucidal activity. However, when the pH is at 4.5 or above, the compositions have little or no virucidal activity.

18. I believe that the data provided in U.S. Patent Application No. 09/795,279 and in Exhibits B and C demonstrate that lower chain alcohols at low concentrations are not virucidal and also that acid solutions above pH 4.5 are not virucidal, but the combination of lower chain alcohols at low concentrations and an acid at a pH between 2.45 and 4.6 provides an effective virucidal composition. Therefore, this combination of non-virucidal agents to produce a virucidal composition demonstrates a synergistic effect.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

JACK KONOWALCHUK

0.6 % glycolic acid mixed with various amounts of ethanol (10 min exposure)

| | pH | | | | |
|---|---|---|---|---|---|
| **% Ethanol** | 2.5 | 3.5 | 4.0 | 4.5 | 5.0 |
| 1% | - | - | - | + | + |
| 5% | - | - | - | - | + |
| 10% | - | - | - | - | -* |

- : No virus growth
+ : Virus growth

* One of two plates had no virus, the other had only one plaque.
Typical plates for compositions that are not active have at least 10 plaques.

US005591442A

# United States Patent [19]

Diehl et al.

[11] Patent Number: 5,591,442

[45] Date of Patent: Jan. 7, 1997

[54] **SKIN ANTISEPTIC AND HAND DISINFECTANT**

[75] Inventors: **Karl H. Diehl**, Norderstedt; **Heinz Eggensperger**, Hamburg; **Peter Goroncy-Bermes**, Ahrensburg; **Peter Oltmanns**; **Susanne Toefke**, both of Hamburg, all of Germany

[73] Assignee: **Reckitt & Colman Inc.**, Montvale, N.J.

[21] Appl. No.: **513,420**

[22] Filed: **Aug. 15, 1995**

**Related U.S. Application Data**

[63] Continuation of Ser. No. 979,715, Nov. 20, 1992, abandoned.

[30] **Foreign Application Priority Data**

Dec. 9, 1991 [DE] Germany ..... 41 40 473.4

[51] **Int. Cl.**[6] ..... **A61K 6/00**; A61K 7/00

[52] **U.S. Cl.** ..... **424/401**; 424/70.1; 424/61; 422/61

[58] **Field of Search** ..... 424/401, 70.1, 424/61

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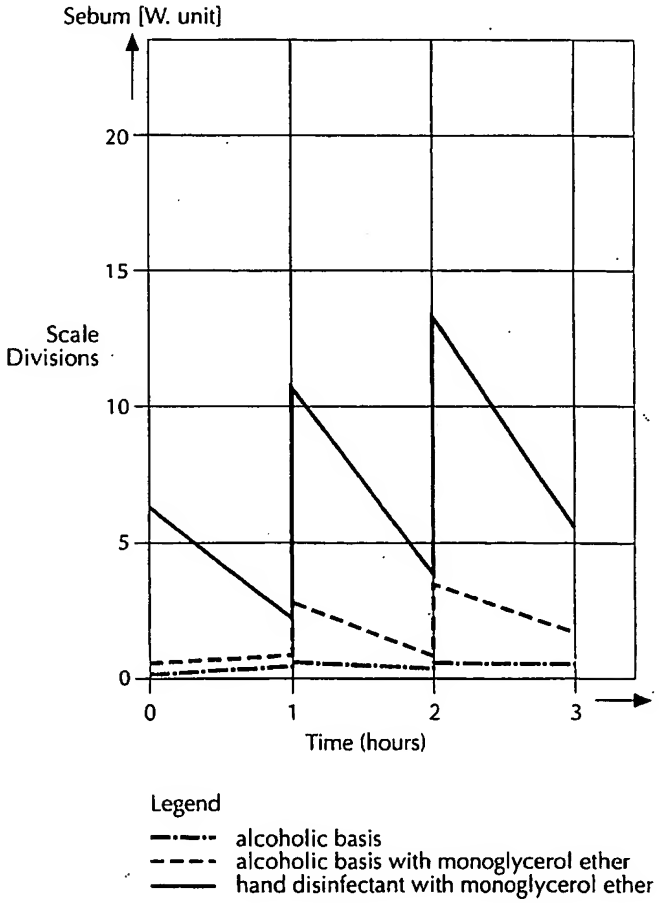
*Primary Examiner*—Thurman K. Page
*Assistant Examiner*—William E. Benston, Jr.
*Attorney, Agent, or Firm*—Frederick H. Rabin; John R. Everett



[57] **ABSTRACT**

A skin antiseptic and hand disinfectant composition is disclosed. The composition comprises a glycerol monoalkyl ether is a glycerol 1-$C_5$–$C_{12}$ alkyl ether and an aliphatic $C_1$–$C_6$ alkyl alcohol.

**10 Claims, 2 Drawing Sheets**



# FIG. 1

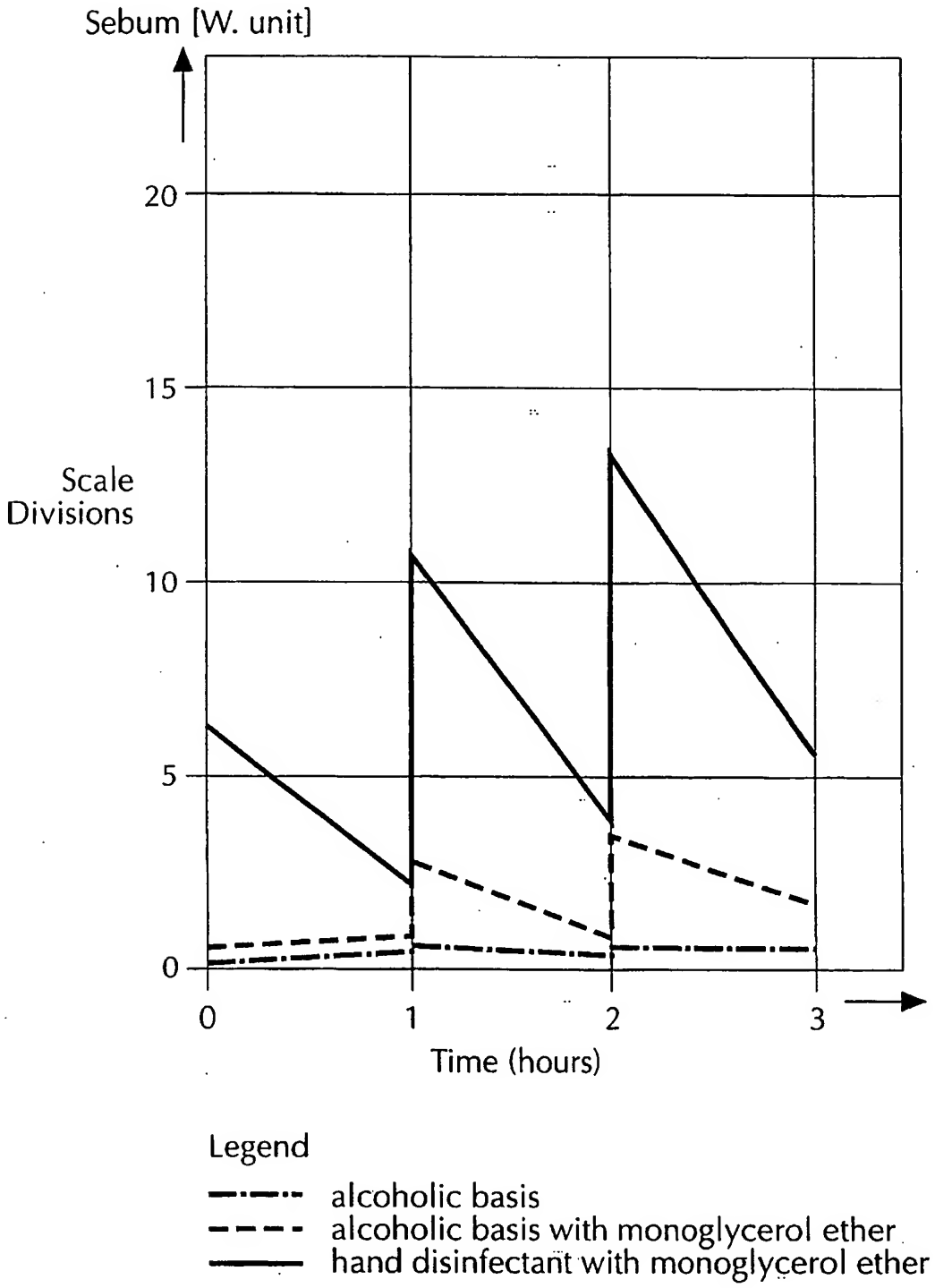
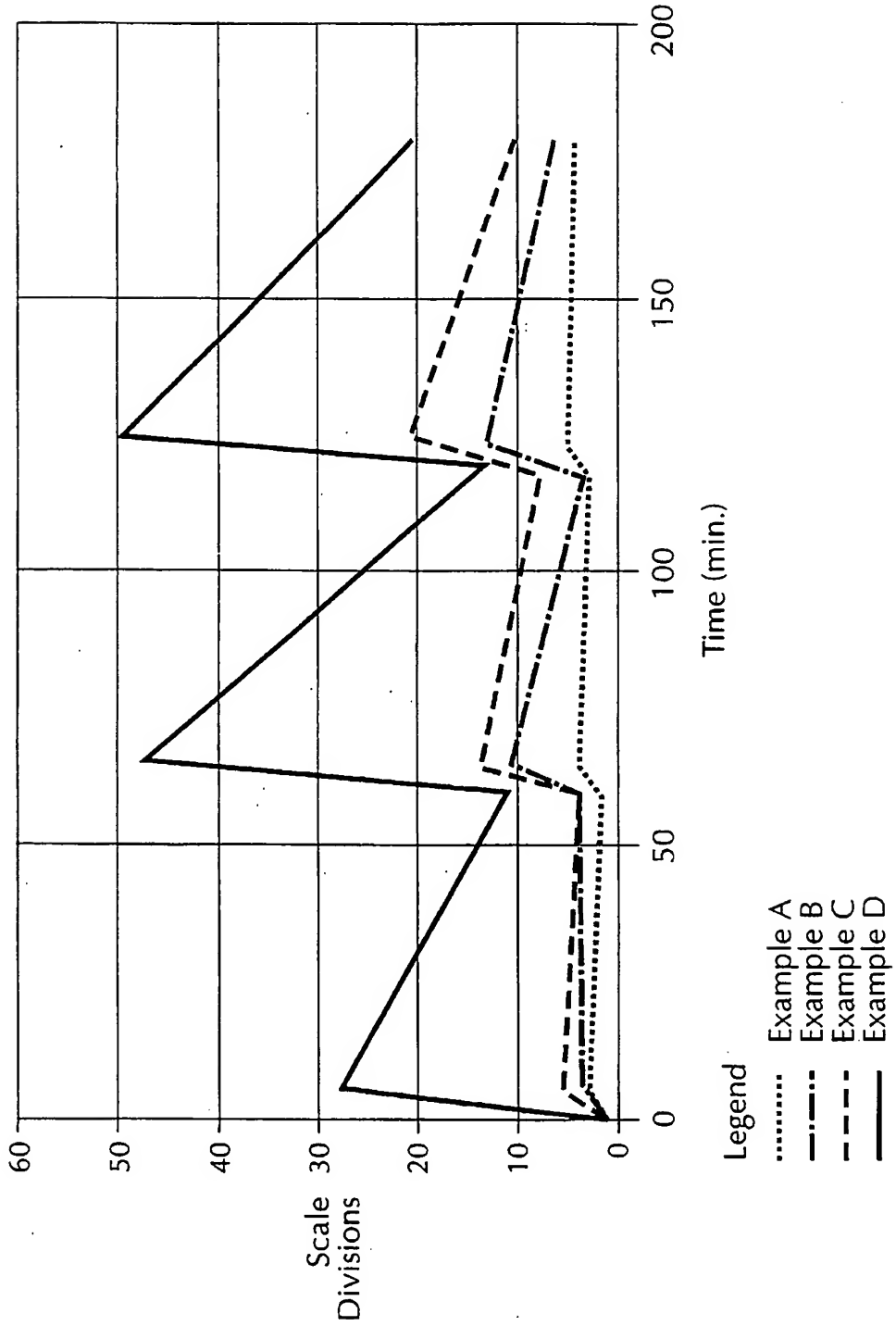

Sebum [W. unit]
20
15
Scale Divisions
10
5
0
0
1
2
3
Time (hours)
Legend
alcoholic basis
alcoholic basis with monoglycerol ether
hand disinfectant with monoglycerol ether

FIG. 2


Scale Divisions
60
50
40
30
20
10
0
0
50
100
150
200
Time (min.)
Legend
Example A
Example B
Example C
Example D

# SKIN ANTISEPTIC AND HAND DISINFECTANT

This application is a continuation, of application No. 07/979715, filed 20 Nov. 1992, now abandoned.

## FIELD OF THE INVENTION

This invention relates to skin antiseptic and hand disinfectant compositions.

## BACKGROUND OF THE INVENTION

Parts of the skin and mucous membranes have long been treated antiseptically prior to surgical intervention, injections or punctures and prior to examinations of body cavities accessible from the outside. Moreover, it is necessary also for those persons who carry out the above treatments and examinations to disinfect their hands before the treatment or examination commences.

For such purposes known compositions with antiseptic action are highly volatile alcohols. However, high alcohol concentrations must be present for effectiveness within a very short contact time (within seconds to a few minutes). The alcohol content is generally more than 50, mostly about 60 to 80% by wt. The alcohols are frequently aliphatic alcohols such as ethanol, 1-propanol and 2-propanol.

In addition to alcohols, hand disinfectants frequently contain other substances such as long-acting active cationic compounds with an antimicrobial action, and skin care components in order to prevent severe drying of the skin. In the case of skin antiseptics containing lipid restorers, the alcohol content must often be higher than in preparations without lipid restoring agents, since said compounds often impair the antiseptic effectiveness of the alcohols or other microbial active ingredients present.

In spite of the lipid restoring skin care components, and given the necessarily frequent use of said preparations, irritation of the skin treated therewith occurs to an increased extent at colder times of the year.

Apart from alcoholic skin antiseptics and hand disinfectants, antimicrobial and disinfectant aqueous emulsions are also known which contain a glycerol monoalkyl ether., e.g. 3-alkoxypropan-1,2-diol (known for example from U.S. Pat. No. DE 649 206). The antimicrobial effectiveness of glycerol ethers (e.g. also known from JP 76-76424) when used alone has proved to be relatively poor in practice, however, so that their use as an active ingredient in skin antiseptics containing substantial quantities of water is completely inadequate without other additives also having an antimicrobial action.

It is an object of the invention to provide a particularly skin-compatible skin antiseptic and hand disinfectant that 1) has a lasting lipid-restoring effect and hence cares for the skin; 2) and yet is a quick-acting antiseptic which capacity and ease of application is not impaired by skin care components and 3) gives a pleasant feel to the skin.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 show the slight increase in oil concentration on skin treated with compositions of the invention.

FIG. 2 illustrates the skin lipid restoring properties of monoalkyl glycerol ethers.



## SUMMARY OF THE INVENTION

This object is achieved by an aqueous composition comprising an alkyl alcohol component and at least one glycerol monoalkyl ether. It was found that this composition exhibits an outstanding antimicrobial effect and at the same time restores the lipid content of the skin.

This is particularly surprising because the glycerol monoalkyl ethers themselves exhibit insufficient antimicrobial action and their lipid restoring properties are hitherto unknown.

The antimicrobial effectiveness of the composition according to the invention is greater than that of the individual alkyl alcohol components or glycerol monoalkyl ether alone. There is, therefore, a genuine synergism.

In view of the synergistic effect of the alkyl alcohol component and the glycerol monoalkyl ether, it is possible to reduce the alkyl alcohol content of the composition compared with that of the well known compositions used as skin antiseptics and hand disinfectants.

The combination of alkyl alcohols in low concentrations with a glycerol monoalkyl ether offers economic advantages by reducing the alcohol content and but also the possibility of improving the compatibility of skin antiseptics and hand disinfectants to skin. The risk of skin irritation is reduced considerably, particularly with frequent use, because of the reduced drying and better lipid-restoring effect of the composition.

As a result of the addition according to the invention of glycerol monoalkyl ether in small quantities to an alkyl alcohol component, it has now proved possible to achieve a sufficiently high efficiency and rapid onset of action and a markedly good lipid-restoring effect.

The glycerol monoalkyl ethers are in particular glycerol 1-($C_5$–$C_{12}$ alkyl) ethers. These include, amongst others, glycerol 1-(2-ethylhexyl) ether, glycerol 1-heptyl ether, glycerol 1-octyl ether, glycerol 1-decyl ether and glycerol 1-dodecyl ether. A preferred compound is glycerol 1-(2-ethylhexyl) ether. These compounds are readily obtainable. Their preparation is described in the literature, e.g. in JP 80-19253, JP 58-134049, U.S. Pat. No. DE 33 43 530 and E. Baer, H. O. L. Fischer in J. Biol. Chem. 140 397 (1941).

The composition according to the invention contains as alkyl alcohol component at least one aliphatic C1–$C_6$ alkyl alcohol. In particular, aliphatic alkyl alcohols such as ethanol, 1-propanol, 2-propanol or a mixture of two or more of said alcohols are suitable for this purpose.

Compositions according to the invention generally contain 15 to 85% by wt., in particular 20 to 50% by wt., preferably 25 to 40% by wt. and more preferably 30 to 35% by wt. alkyl alcohol component, 0.1 to 5% by wt., preferably 0.5 to 2.5% by wt. and more preferably 0.5 to 1.5% by wt. glycerol monoalkyl ether and water. In particular preference, the composition contains 35% by wt. alkyl alcohol component, 1% by wt. glycerol monoalkyl ether and water.

Moreover, the composition of the invention may contain one or several other compounds with antiseptic properties action, dye and/or perfume and other customary additives and auxiliaries such as surfactants. In contrast to the additives such as skin care additives optionally present in well known skin antiseptics and hand disinfectants, other additives present in the composition according to the invention do not impair the antiseptic action. As a result, the disadvantage of having to increase the alkyl alcohol concentration in the presence of such additives such as skin care components, for example, is also avoided.

The following examples 1 to 6 show possible formulations for compositions according to the invention:

| | |
|---|---|
| Example 1 | |
| Ethanol | 85 % |
| o-phenylphenol | 0.1 % |
| Glycerol 1-(2-ethylhexyl) ether | 1.5 % |
| Demineralized water | q.s. 100 % |
| Dye | |
| Perfume | |
| Example 2 | |
| 1-Propanol | 50 % |
| 2-Propanol | 20 % |
| Glycerol 1-(2-ethylhexyl) ether | 1 % |
| Demineralized water | q.s. 100 % |
| Dye | |
| Perfume | |
| Example 3 | |
| 2-Propanol | 70 % |
| Ethanol | 10 % |
| Glycerol 1-heptyl ether | 1.3 % |
| Demineralized water | q.s. 100 % |
| Dye | |
| Perfume | |
| Example 4 | |
| Ethanol | 45 % |
| Benzethonium chloride | 0.6 % |
| Glycerol 1-decyl ether | 0.5 % |
| Demineralized water | q.s. 100 % |
| Dye | |
| Perfume | |
| Example 5 | |
| 2-Propanol | 35 % |
| Lactic acid | 0.3 % |
| Glycerol 1-octyl ether | 0.8 % |
| Demineralized water | q.s. 100 % |
| Dye | |
| Perfume | |
| Example 6 | |
| 1-Propanol | 55 % |
| Ethanol | 15 % |
| Glycerol 1-(2-ethylhexyl) ether | 1.2 % |
| Demineralized water | q.s. 100 % |
| Dye | |
| Perfume | |

As has already been mentioned, alkyl alcohols are very effective against microorganisms, the action commencing within a relatively short time (a few minutes), but high concentrations must be chosen in order to achieve very short contact times (1 minute or less).

In a quantitative suspension test, the relationship was determined between the reaction time and the alcohol concentration with and without glycerol monoalkyl ether. As the results in Table 1 show, a reaction time of 120 seconds was required for a reduction in the bacterial count of Pseudomonas aeruginosa by more than 5 log steps with an alcohol concentration of 35% by wt., or at least a concentration of 40% ethanol was required to halve this reaction time to 60 seconds or less. In contrast, when a composition according to the invention is used, e.g. the addition of a glycerol monoalkyl ether in small quantities (1% by wt.) to 35% ethanol, a reaction time of only 60 seconds is sufficient to obtain the same reduction in the bacterial count. With the same alcohol concentration, therefore, the contact time could be reduced to one half or, looked at from the other way round, the ethanol content could be reduced by 5% by wt. (a reduction of 12.5%) with the same contact time.

TABLE I

Reduction in Bacterial Count of *S. aureus* and *P. aeruginosa* in the Quantitative Suspension Test

| Ethanol concentration in wt % | Exposure time in seconds 15 | 30 | 60 | 120 |
|---|---|---|---|---|
| *P aeruginosa* | | | | |
| 20% | 0 | 0 | 0 | 0 |
| 30% | 0 | 0 | 0 | 0 |
| 35% | 1.05 | 1.60 | 2.55 | ≧5.48 |
| 40% | ≧5.49 | ≧5.43 | ≧5.40 | ≧5.48 |
| 35% ethanol + 1% glycerol ether | | | ≧5.38 | |
| *S. aureus* | | | | |
| 35% | 0 | 0 | 0 | 1.16 |
| 40% | 0 | 1.11 | 2.04 | 5.09 |
| 35% ethanol + 1% glycerol ether | | | ≧5.44 | |

Even when 40% ethanol was used, a contact time of 120 seconds was required to reduce the bacterial count of Staphylococcus aureus by >5 log steps. On the other hand, the same reduction in the bacterial count could be achieved in 60 seconds with 35% ethanol in combination with 1% glycerol ether. In this case, therefore, both a reduction in the exposure time and a reduction in the alcohol concentration could be achieved.

The results of the above quantitative suspension tests was confirmed by tests on the skin of the lower arms of voluntary subjects. Table II shows the reduction factors that were obtained with 70% isopropanol (reference solution according to the DGHM* (German Society for Hygiene and Microbiology) code of practice for testing and evaluating skin disinfectants, situation on 1.1.91, Zbl. Hyg. 192 (1991), page 99–103) or 35% ethanol, and with a combination of 35% ethanol and 1% glycerol monoalkyl ether after a contact time of 30, 60 and 120 seconds. The results show that a combination of ethanol and glycerol ethylhexyl ether produces an effect which, on the one hand, goes beyond the extent to be expected and, on the other hand, is comparable with the effect of 70% isopropanol in spite of an alcohol content that has been reduced by one half. Since glycerol ether alone has antimicrobial action only to a minor extent, a synergistic effect must be assumed.

TABLE II

Reduction in Bacterial Count of *Micrococcus luteus* on Skin Williamson and Kligman Test

| | Reduction factor |
|---|---|
| Reaction time: 30 s | |
| 70% Isopropanol | 2.11 |
| 35% Ethanol | 0.37 |
| 35% Ethanol + 1% glycerol ethylhexyl ether | 1.62 |
| Reaction time: 60 s | |
| 70% Isopropanol | 2.30 |
| 35% Ethanol | 0.61 |
| 35% Ethanol + 1% glycerol ethylhexyl ether | 1.71 |
| Reaction time: 120 s | |
| 70% Isopropanol | 2.37 |
| 35% Ethanol | 0.65 |
| 35% Ethanol + 1% glycerol ethylhexyl ether | 1.79 |

Without the addition of alcohol, glycerol monoalkyl ethers as pure substances are sparingly soluble in water.

Aqueous suspensions of the ethers exhibit an insufficient microbial action. The above compositions according to Tables I and II were tested with the active ingredients ethanol and isopropanol individually and the combination ethanol and glycerol ether in aqueous solution. The test organisms used to test the microbicidal effect were *Staphylococcus aureus, Pseudomonas aeruginosa* and *Micrococcus luteus*. The bactericidal effect was determined in the quantitative suspension test according to the code of practice of the German Society for Hygiene and Microbiology (DGHM) and in the Williamson and Kligmann skin test.

A particular advantage of using glycerol ether arises from the fact that it exhibits outstanding lipid-restoring properties particularly in the low concentrations in which it contributes to the increase in the antimicrobial effectiveness. This is wholly in line with modern formulations in which, for reasons of better compatibility, as few as possible different ingredients should be used.

It can be shown in skin measurements that when used through the day the glycerol ether brings about a slight increase in the concentration of the lipid content on the skin, without this making itself noticeable in a negative way as a greasy film (see FIG. 1).

A sebumeter SM 810 PC from Courage and Khazaka, Cologne, was used as measuring apparatus. Every hour, 3 mL of hand disinfectant were rubbed for 90 seconds into the hands which had not been treated beforehand. After 3 hours, the test was halted. The measurements were carried out directly before and after each application, the back of each hand being measured twice and the palm of each hand being measured once (skin lipid measurements).

In order to demonstrate the superior lipid-restoring property of the glycerol ether-containing compositions according to the invention in comparison with well known hand disinfectants, measurements of the skin oil content were carried out with the sebumeter. The measuring conditions and the apparatus were the same as above.

The following formulations were tested on a comparative basis:

Formulation A

60% ethanol
20% i-propanol
q.s. 100% water

Formulation B

60% ethanol
20% i-propanol
1% glycerol 1-(2-ethylhexyl) ether
q.s. 100% water

Formulation C

60% ethanol
20% i-propanol
1% myristyl alcohol
q.s. 100% water

Formulation D

60% ethanol
20% i-propanol
1% myristyl alcohol
1% glycerol 1-(2-ethylhexyl) ether
q.s. 100% water

The graphical plot of the sebumeter values obtained (see FIG. 2) illustrates the superior lipid-restoring effect brought about by the glycerol ether. In order to obtain a better comparison with the hand disinfectants known hitherto, a total alcohol content of 80% was chosen in all the formulations, which is customary in commercial preparations.

After the application of formulation B, there remains a higher skin lipid content after each application compared with the application of formulation A, the difference between the formulations being that formulation A contains no glycerol ether.

This effect becomes particularly pronounced in a comparison of formulations C and D. With formulation D, markedly higher skin lipid contents are achieved than with formulation C which already contains a well known lipid-restoring component (myristyl alcohol). The higher skin lipid content, which is obtained after application of formulation D, falls markedly within an hour of application but there remains a higher lipid content than after application of formulation C, particularly after repeated application.

The increased lipid-restoring property of the glycerol ethers in the compositions according to the invention is subjectively discernable by a pleasant feel to the skin that remains after application, which can be described with the adjectives velvety, soft, smooth, non-greasy and non-sticky.

The compositions according to the invention can be prepared by mixing the individual components together successively, if necessary with heating. No particular order need be adhered to during this process.

Seen as a whole, therefore, the compositions according to the invention are especially suitable as skin antiseptics and hand disinfectants.

The invention has been described in detail with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

We claim:

1. A method for cleaning and disinfecting the skin which comprises applying to the skin an aqueous antiseptic and disinfectant composition comprising from 15 to 85% by weight of an aliphatic $C_1$–$C_6$ alkyl alcohol and from 0.1 to 5% by weight of a glycerol 1-($C_5$–$C_{12}$ alkyl) ether.

2. A skin antiseptic and hand disinfectant composition comprising a glycerol 1-($C_5$–$C_{12}$ alkyl) ether and an aliphatic $C_1$–$C_6$ alkyl alcohol.

3. A composition according to claim 2 wherein the glycerol ether is selected from the group consisting of glycerol 1-(2-ethylhexyl) ether, glycerol 1-heptyl ether, glycerol 1-octyl ether, glycerol 1-decyl ether and glycerol 1-dodecyl ether.

4. A composition according to claim 2 wherein the alkyl alcohol component contains ethanol, 1-propanol, 2-propanol or a mixture of two or more of said alcohols.

5. A composition according to claim 2 containing 15 to 85% by wt. alkyl alcohol and 0.1 to 5% by wt glycerol monoalkyl ether.

6. A composition according to claim 2 containing 20 to 50% by wt. of alkyl alcohol and 0.5–2.5% by wt. glycerol monoalkyl ether.

7. A composition according to claim 2 containing 25–40% by wt. alkyl alcohol and 0.5–1.5% by wt. glycerol monoalkyl ether.

8. A composition according to claim 2 containing 30–35% by wt. alkyl alcohol and 1% by wt. glycerol monoalkyl ether.

9. A composition according to claim 2 containing 35% by wt. alkyl alcohol and 1% by wt. glycerol monoalkyl ether.

10. The composition of claim 3 wherein the ether is 1-(2-ethylhexyl)glycerin ether.

* * * * *

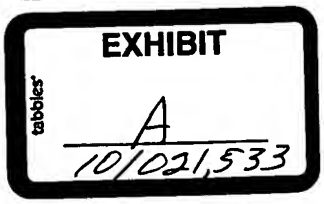
US006080417A

# United States Patent [19]

Kramer et al.

[11] Patent Number: 6,080,417

[45] Date of Patent: Jun. 27, 2000




[54] **HAND DISINFECTANT**

[75] Inventors: **Axel Kramer**; **Leopold Döhner**, both of Greifswald, Germany

[73] Assignee: **Antiseptica Chemisch-Pharmazeutische Produkte GmbH**, Germany

[21] Appl. No.: **08/952,685**

[22] PCT Filed: **Jan. 31, 1997**

[86] PCT No.: **PCT/EP97/00417**

§ 371 Date: **Aug. 31, 1998**

§ 102(e) Date: **Aug. 31, 1998**

[87] PCT Pub. No.: **WO97/35475**

PCT Pub. Date: **Oct. 2, 1997**

[30] **Foreign Application Priority Data**

Mar. 27, 1996 [DE] Germany ..... 196 12 057

[51] **Int. Cl.**[7] ..... **A01N 25/00**; A01N 31/00; A01N 41/10; A61K 31/075

[52] **U.S. Cl.** ..... **424/405**; 424/400; 424/616; 514/706; 514/709; 514/714

[58] **Field of Search** ..... 424/400, 405, 424/616; 514/706, 709, 714

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*Primary Examiner*—S. Mark Clardy
*Assistant Examiner*—Kathryne E. Shelborne
*Attorney, Agent, or Firm*—Pillsbury, Madison & Sutro LLP

[57] **ABSTRACT**

The invention concerns a hand disinfectant based on lower alcohols and is characterized by an aqueous solution of lower alcohols with synergists, the solution having a flash point of more than 21° C.

**11 Claims, No Drawings**

# HAND DISINFECTANT

Hand disinfectants containing one or more lower alcohols, such as ethanol, isopropanol or n-propanol, are widely known. As a rule, they are aqueous solutions with an alcohol content of 70 to 80 weight %, and optionally other compounds that have microbicidal action are added. These known hand disinfectants meet the requirements, formulated by DGHM, for the germicidal effect against bacteria, including mycobacteria, and fungi.

Increasingly, the viral effectiveness of hand disinfectants based on alcohol has lately been discussed. Their virus inactivation, particularly against highly resistant types of virus, such as polio, does not meet all demands; for instance, it is known that polio viruses for instance can be inactivated only with a very high percentage of ethanol, and so the usual concentration of between about 70 and 80 weight % is not adequate for this purpose. Hence hand disinfectants based on 90 to 95 weight % ethanol are already on the market. Although these disinfectants meet the demands for virus inactivation made by the DVV, they have some decisive disadvantages. The most important disadvantage is that the flash point of such mixtures with a high ethanol content is below 21° C., which makes the preparations subject to the Code on Flammable Liquids in hazard class B1, and special regulations must be obeyed regarding shipping, storage, bottling, and the like. This is a particular burden for hospitals and large medical practices, because large quantities of disinfectants are needed, and in general the supply rooms of the applicable institution are not set up for storing relatively large quantities of flammable liquids. Another disadvantage is that disinfectants with such a high alcohol content, when used for disinfecting hands, dry the skin to an extraordinary degree, with all the attendant, well-known side effects.

There is therefore still an urgent need for hand disinfectants with good virus inactivation effectiveness even for known resistant types of virus, but which do not come under hazard class B1 of the Code on Flammable Liquids and which moreover are readily tolerated.

To attain this object, hand disinfectants on the basis of lower alcohols are proposed, which are characterized in that they contain lower alcohols together with synergists in an aqueous solution and have a flash point above 21° C.

Surprisingly, it has been found that instead of the previously conventional high-percentage ethanol solutions for viricidal hand disinfection, preparations that have a substantially lower alcohol content in the form of lower alcohols can also be used, if they contain mixtures of synergists that promote the viricidal action or virus inactivation of the alcohols, and hence mixtures can be prepared that are no longer in hazard class B1 of the Code on Flammable Liquids. Those whose use is preferred are the lower alcohols, that is, ethanol, isopropanol or n-propanol, which in high concentration have flash points below 21° C., but which are preferably used in such an aqueous solution that the total alcohol content is between about 50 and 60 volume %.

To increase the viricidal or virus-inactivating action, diols are used, specifically preferably those with a chain length of from 3 to 5 carbon atoms. Propanediols are especially suitable, and both positional isomers, that is, 1,2-propanediol and 1,3-propanediol can be used. 1,2-Propanediol is considered safe, although recent studies have indicated that the toxicity may be somewhat higher than in the 1,2-isomer. Along with the propanediols, butanediols can also be considered, and specifically all the positional isomers, but 1,3-butanediol is preferred, because the most toxicological data is available for it.



The diols have a certain bacteriostatic action, and moreover they are used in the foods industry against fungi, for example, and especially against yeasts. The concentration of the diols may be low and ranges from about 3 to about 10 volume Besides the diols, other additives can also be considered, specifically hydrogen peroxide in a 1 to 3% concentration and sodium alkane sulfonates (E30), that is, essentially secondary sulfonic acids with a chain length of the alkyl group of between about 12 and 18 carbon atoms. Alkane sulfonates are environmentally tolerable anionic surfactants which exhibit a strong virus-inactivating effect. The concentration of the alkane sulfonates is in the range frog about 0.2 to about 0.7 weight %.

The substance known as sodium rhodanide, NaSCN, is another highly effective synergistic compound. It is indeed known that thiocyanic acid and its salts are microbicidally effective, but so far this activity has hardly been utilized for disinfecting purposes. The concentration of rhodanide should be between about 1 and 3 weight %.

The mixtures according to the invention are adjusted to be acidic, which can be done by adding physiologically safe organic acids that are easy on the skin, in a concentration of about 0.001 to 0.005 mole %. Preferably, citric acid, tartaric acid, malonic acid or malic acid is used, because these acids have no odor of their own. However, other toxigologically unobjectionable acids may also be used, such as lactic acid, acetic acid, formic acid or propionic acid, and similar compounds.

The mixtures according to the invention have flash points above 21° C. and are therefore not subject to the more-stringent requirements made of substances in hazard class B1. They are markedly easier on the skin than preparations with a very high content of alcohols, because drying of the skin does not ensue to the same degree as with high-alcohol solutions. Their effectiveness against bacteria, yeasts and fungi meets the regulations of the DVV or of BIFAM. But in terms of virus-inactivating effectiveness, they also meet the requirements of the DGHM.

The preparation of the disinfectants of the invention is done in a manner known per se, by dissolving the usually solid organic acids and by dissolving the synergistically active compounds, such as alkane sulfonates or rhodanide, in some of the total amount of water needed, mixing the liquid alcohols, and adding the total amount of water needed.

The invention will be described in further detail below in terms of the Examples:

## EXAMPLE 1

6 l of 96% ethanol are mixed with 500 ml of 1,2-propanediol and 500 ml of 1,3-butanediol and mixed carefully. Into this mixture, an aqueous solution of 200 ml of 1-molar citric acid is worked in, and the mixture is then diluted with 2.8 l of double-distilled water. The flash point, determined in accordance with DIN 51755, is 22.5° C.

## EXAMPLE 2

6 l of 96% ethanol, as indicated in Example 1, are mixed with 500 ml of 1,2-propanediol and 500 ml of 1,3-butanediol and 200 ml of a 1-molar tartaric acid solution and diluted with 2.8 l of distilled water. The flash point, determined in accordance with DIN 51755, is 23.0° C.

## EXAMPLE 3

As described in Example 1, 6 l of 96% ethanol, 1 l of 1,2-propanediol, 1 l of 1,3-butanediol and 200 ml of a 1-molar citric acid are mixed and water is added to make up 10 l.

EXAMPLE 4

6 l of 96% ethanol, 3 l of 1,2-propanediol, and 200 ml of 1-molar malic acid are mixed and water is added to make up 10 l.

EXAMPLE 5

6 l of 96% ethanol are mixed with 1 l n-propanol, 500 ml of 1,2-propanediol and 500 ml of 1,3-butanediol and mixed carefully. Into this mixture, an aqueous solution of 200 ml of 1-molar citric acid is worked in, and the solution is then diluted with 1.8 l of double-distilled water.

The flash point, determined in accordance with DIN 51755, is 22° C.

EXAMPLE 6

6 l of 96% ethanol are mixed with 500 ml n-propanol, 500 ml of isopropanol, 500 ml of 1,2-propanediol and 500 ml of 1,3-butanediol and mixed. Into this mixture, 200 ml of a 1-molar malic acid solution is then worked in, and the solution is then diluted with 1.8 l of double-distilled water.

The flash point, determined in accordance with DIN 51755, is 21.5° C.

EXAMPLE 7

6 l of 96% ethanol are mixed with 200 ml n-propanol, 700 ml isopropanol, 500 ml of 1,2-propanediol and 500 ml of 1,3-butanediol and mixed. Into this mixture, 200 ml of 1-molar citric acid is then worked in, and the solution is then diluted with 1.9 l of double-distilled water.

The flash point, determined in accordance with DIN 51755, is 21° C.

We claim:

1. A hand disinfectant based on lower alcohols which comprises an aqueous solution of lower alcohols along with one or more diols and one or more synergists selected from the group consisting of hydrogen peroxide, alkane sulfonates and salts of thiocyanic acid, said disinfectant having a flash point above 21° C.

2. The hand disinfectant of claim 1 which contains ethanol.

3. The hand disinfectant of claim 2 which has an ethanol content between about 50 and about 60 volume %.

4. The hand disinfectant of claim 3 which has an ethanol content of about 57 volume %.

5. The hand disinfectant of claim 1 which contains propanediol.

6. The hand disinfectant of claim 1 which contains butanediol.

7. The hand disinfectant of claim 1 which contains 1,2-propanediol.

8. The hand disinfectant of claim 1 which contains 1,3-butanediol.

9. The hand disinfectant of claim 1 which contains approximately 5–10 volume % diols, 1–3 weight % hydrogen peroxide or salts of thiocyanic acid, and/or 0.2 to 0.7 weight % alkane sulfonates.

10. The hand disinfectant of claim 1 which contains at least one physiologically safe organic acid.

11. The hand disinfectant of claim 1 which contains between about 0.01 and 0.05 mole % organic acids.

0.0001 0.005

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UNITED STATES PATENT AND TRADEMARK OFFICE

# CERTIFICATE OF CORRECTION

| | | |
|---|---|---|
| PATENT NO. | : | 6,080,417 |
| DATED | : | June 27, 2000 |
| INVENTOR(s) | : | KRAMER, Axel and DOHNER, Leopold |

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 4, line 31, please change "about 0.01 and 0.05 mole % organic acids" to --about 0.001 and 0.005 mole % organic acids--.

Signed and Sealed this

Eighth Day of May, 2001

*Attest:*

Nicholas P. Godici

NICHOLAS P. GODICI

*Attesting Officer*

*Acting Director of the United States Patent and Trademark Office*

*Journal of Hospital Infection* (1980) 1, 321–325

# The action of alcohols on rotavirus, astrovirus and enterovirus

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**Summary:** The virucidal effects of a series of five alcohols on rotavirus, astrovirus and echovirus 11 were studied. The reaction time between the alcohol and virus was one minute, a time for which a hand disinfectant might be applied. The efficacy of the alcohols rose with the concentration used. Forty per cent concentrations of the higher alcohols (propan-1-ol, propan-2-ol and butan-2-ol) caused at least a $10^4$-fold drop in rotavirus titre. Methanol and ethanol were not quite as effective against rotavirus, but were the only alcohols of those tested that reduced the titres of the more resistant astrovirus and echovirus 11, and then only when used at high concentrations. Preparations incorporating 90 per cent ethanol are recommended as hand disinfectants with a broad virucidal activity.

## Introduction

Much has been written on the action of disinfectants against pathogenic bacteria and standard procedures are now used to evaluate them. For disinfecting skin Lowbury, Lilly & Ayliffe (1974) have clearly shown the effectiveness of an alcoholic solution of chlorhexidine in reducing the viable bacterial counts of surgeons' hands. The virucidal activity of hand disinfectants has, however, seldom been studied in spite of reports that enteroviruses (Melnick, 1976) and rhinoviruses (Hendley, Wenzel & Gwattney, 1973) may be spread on hands.

In hospitals, babies in neonatal units are especially subject to cross-infection which may be endemic, as was the the case for rotaviruses reported by Chrystie, Totterdall & Banatvala (1978) or take the form of epidemics, an example of which was the Cambridge echovirus 11 outbreak reported by Nagington, Wreghitt, Gandy, Robertson & Berry (1978). The relative effects of various disinfectants on echovirus 11 have been studied by Drulak, Wallbank & Lebtag (1978), and they found that 76 per cent (v/v) ethyl alcohol caused a $10^6$ reduction in virus titre following 20 s exposure. Our studies (Kurtz, 1979), supported this finding, but also showed that isopropyl alcohol at a similar dilution failed to lower the virus count following 1 min contact, a time for which a hand disinfectant might be applied.

We report now the reduction in infectivity of rotavirus and astrovirus, both of which are common causes of infantile gastroenteritis, as well as echovirus 11, following 1 min contact with various alcohols.

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## Materials and methods

The alcohols tested were methanol, ethanol, propan-1-ol, propan-2-ol and butan-2-ol (B.D.H. Chemicals Ltd).

### *Tests with rotavirus*

An adapted strain of bovine rotavirus (kindly provided by Dr Bridger, ARC, Compton, Berkshire), grown in $LLCMK_2$ cells was ampouled and stored at −70°C. The activity of the alcohols was tested as follows: to 0·3 ml volumes of the virus containing fluid were added 0·7 ml volumes of different dilutions of the alcohols, to give final concentrations between 20 and 70 per cent. After 1 min, 2 ml of serum-free 199 medium containing 0·2 μg/ml trypsin (199T), which was incorporated into the medium to enhance the infectivity of rotaviruses in tissue culture (Almeida, Hall, Banatvala, Totterdell & Chrystie, 1978), was added to the mixture. Immediately a further 1:10 dilution of this was made in 199T. For a control, 0·3 ml of the virus containing fluid was held with 0·7 ml phosphate buffered saline (PBS) for 1 min and then after adding 2 ml of 199T the 10-fold dilution was made in 199T containing 2·5 per cent of the alcohol under test. The alcohol was added to the control at this stage to match the effect of the residual alcohol in the test which might continue to affect the virus or the cells. Virus infectivity was assayed in $LLCMK_2$ cells. 2 ml aliquots of the above dilutions were put onto coverslips in flat-bottomed tubes. The tubes were centrifuged at 3000 r/min for 1 h at 35°C. The medium was replaced with 199T and the tubes incubated at 37°C for 18–24 h. After fixing in acetone, the coverslips were treated with a bovine anti-rotavirus serum and then fluorescein labelled rabbit anti-bovine globulin (Wellcome Research Laboratories Ltd.). The coverslips were examined with a Vickers incident light fluorescent microscope and the fluorescing cells counted. From these the number of infective doses of virus in the reaction mixtures were calculated. Further tests to determine the effect of organic material on the activity of the alcohols were done using equal volumes of virus and sterilized faeces.

### *Tests with astrovirus*

A human faecal extract (10 per cent) containing large numbers of astroviruses was used as the virus source. Virucidal activity was measured by adding to 0·1 ml volumes of the faecal extract 0·9 ml of dilutions of the alcohols under test. After one minute reaction time, 19 ml of 199 medium containing 20 per cent foetal calf serum (199S) was added, and further 10-fold dilutions made in the same medium. For a control, similar volumes of faecal extract and PBS were mixed and held for one minute after which the initial dilution was made in 199S containing 4·5 per cent of the alcohol under test. Aliquots of 1 ml of dilutions were then put onto monolayers of human embryo kidney cells grown on coverslips. After one hour the inoculum was removed and replaced by 199S. The coverslips were then incubated 18–24 h at 37°C. After fixing in acetone the coverslips were treated with a human anti-astrovirus serum, stained with a fluorescein labelled rabbit anti-human globulin (Wellcome Research Laboratories Ltd), and the fluorescing cells counted as above.

*Tests with echovirus* 11

A recently isolated strain of echovirus 11 grown in human embryo lung fibroblasts (HEL) and stored at −70°C was used. The method originally described by Drulak *et al.* (1978), who found that 17·5 per cent skimmed milk effectively neutralized the effect of a diverse group of disinfectants, was adapted (Kurtz, 1979), and was briefly as follows: to 0·05 ml of the virus and 0·05 ml of calf serum were added 0·4 ml volumes of dilutions of the alcohol to be tested. The concentration of alcohol in the reaction mixture was 4/5th of its initial concentration. After 1 min reaction time, 4·5 ml of skimmed milk (17·5 g per 100 ml) was added to neutralize the effect of the alcohol. A two-fold dilution and then serial 10-fold dilutions were made in Eagles MEM, supplemented with 2 per cent calf serum. Volumes of 1 ml of these dilutions were inoculated onto HEL monolayers and observed for up to 5 days for cytopathic effect (CPE). Virus titres were recorded as the greatest dilution ($log_{10}$) showing any CPE and the number of infective units/ml of reaction mixture calculated. For a control, to 0·1 ml of the virus–serum mixture was added 0·4 ml PBS and the skimmed milk added one minute later contained 9·5 per cent of the alcohol being tested.

## Results

Table I shows that rotavirus titres were not affected by 20 per cent methanol or ethanol, although there was a 1–3 $log_{10}$ drop when the higher alcohols were used at this concentration. With 30 per cent concentrations, all the alcohols tested, except

Table I. *Bovine rotavirus titres after alcohol treatment (1-min holding time)*

| | Alcohol in reaction mixture (per cent) | | | | |
|---|---|---|---|---|---|
| | 0 (control) | 20 | 30 | 40 | 50 |
| Methanol | 5·9* | 5·5 | 5·7 | 3·6 | 2·2 |
| + faeces | 5·3 | | | 3·3 | 2·3 |
| Ethanol | 5·9 | 5·9 | 2·7 | <1·9 | |
| + faeces | 5·7 | | 1·9 | <1·9 | |
| Propan-1-ol | 5·9 | 2·9 | 2·2 | <1·9 | |
| + faeces | 5·2 | | 1·9 | | <1·9 |
| Propan-2-ol | 5·8 | 4·3 | 2·3 | <1·9 | |
| + faeces | 5·5 | | 2·9 | | |
| Butan-2-ol | 5·9 | 2·9 | <1·9 | | |
| + faeces | 5·3 | | 1·9 | <1·9 | |

*$Log_{10}$ infective units/ml reaction mixture.
Each entry is the mean of four experiments.

methanol, gave a 3–4 $log_{10}$ reduction in titre. Rotavirus was still present after treatment with 50 per cent methanol, but the other alcohols all reduced the virus count below the minimum detectable in this test system (75 infective units/1 ml reaction mixture) when used at a 40 per cent concentration. Higher concentrations of all the alcohols (70 per cent and 90 per cent) again lowered the virus titre below detectable numbers. It can also be seen that the presence of faecal material caused only a slight reduction in the effectiveness of the alcohols.

Table II. *Astrovirus titre after alcohol treatment (1 min holding time)*

| | Alcohol in reaction mixture (per cent) | | |
|---|---|---|---|
| | 0 (control) | 70 | 90 |
| Methanol | 4·0* | 1·0 | <1·0 |
| + faeces | 4·0 | 1·0 | <1·0 |
| Ethanol | 5·7 | 4·3 | 1·7 |
| + faeces | 4·5 | | 3·8 |
| Propan-1-ol | 5·3 | | 5·3 |
| Propan-2-ol | 4·8 | | 4·7 |
| Butan-2-ol | 4·8 | | 4·8 |

*$Log_{10}$ infective units/ml of reaction mixture.
Each entry is the mean of four experiments.

None of the alcohols used at 50 per cent concentration had any effect on astrovirus or echovirus 11. Table II shows the titres of astrovirus following one minute contact with 70 and 90 per cent concentrations. Propan-1-ol, propan-2-ol and butan-2-ol all failed to reduce the virus titre even when used at 90 per cent concentration. Seventy per cent ethanol produced a 1 $log_{10}$ drop in astrovirus count and a 4 $log_{10}$ drop was obtained with 90 per cent. Methanol was the most effective of all the alcohols tested causing a 3 $log_{10}$ drop in astrovirus titre when used at 70 per cent concentration. Ninety per cent methanol reduced the virus count below the limit of sensitivity of the test (10 infective units/ml reaction mixture). The presence of faecal material adversely affected the action of 90 per cent ethanol but interfered less with methanol.

Echovirus 11 was resistant to the three higher alcohols at the highest concentration tested in the reaction mixture. Seventy-six per cent ethanol caused a 3 $log_{10}$ reduction in virus count and at least a 4 $log_{10}$ drop in titre followed the use of 76 per cent methanol (Table III).

## Discussion

Previous studies on the action of disinfectants against the lamb rotavirus (Snodgrass & Herring, 1977) were concerned with those agents suitable for use on inanimate objects. Contaminated fomites may represent one route of cross-infection, but person to person transmission via hands is at least as important Hendley *et al.*, 1973). We have shown that rotaviruses are relatively easily inactivated by the al-

Table III. *Echovirus titre after alcohol treatment (1-min holding time)*

| | Alcohol in reaction mixture (per cent) | | |
|---|---|---|---|
| | 0 (control) | 60 | 76 |
| Methanol | 6·3* | 4·3–5·3 | 1·3–2·3 |
| Ethanol | 6·3 | 6·3 | 3·3 |
| Propan-1-ol | 6·3 | 6·3 | 6·3 |
| Propan-2-ol | 6·3 | 6·3 | 6·3 |
| Butan-2-ol | 6·3 | 6·3 | 6·3 |

*$Log_{10}$ infective units/ml reaction mixture.
Each entry is the mean of eight experiments.

cohols tested. Tests performed on this virus, even in the presence of particulate faecal matter showed that a 50 per cent concentration of any of the alcohols except methanol completely removed infective viruses as detected by this system. Methanol was the least active of the alcohols tested but even it caused a 3 $\log_{10}$ reduction in virus titre when used at 50 per cent.

The astrovirus and echovirus 11—a representative of the enterovirus genus—were considerably more resistant to alcoholic inactivation. Curiously, the three higher alcohols—propan-1-ol, propan-2-ol and butan-2-ol—which were the most active against rotavirus, failed to reduce the infectivity of astrovirus or echovirus 11, even at 90 per cent concentration. A 4 $\log_{10}$ fall in astrovirus titre was obtained with 90 per cent ethanol and methanol.

The effects of the alcohols on the enterovirus were similar to their effects on astrovirus. Against both small round viruses, methanol was the most active, although it had performed least well against rotaviruses.

A consideration of these results suggests that in situations where virus infections may be disseminated via the hands, the use of an alcoholic disinfectant in addition to, or as a partial substitute for handwashing, should help limit their spread. For this purpose the broadest spectrum of virucidal activity is desirable, and this, we have shown, is achieved with high concentrations (90 per cent) of either ethanol or methanol.

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